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13. ABSTRACT (Maximum 200 words) We have, to date, 48 Hereditary Breast/Ovarian Cancer families with an identified BRCA1 or BRCA2 genetic mutation, wherein about 211 breast cancer patients' slides and tissue blocks are being or have been retrieved together with medical histories, pedigree relationships, survival data, and 102 completed risk factor questionnaires. Previous findings have shown that BRCA1-related and other HBC patients presented at lower stage ($p = 0.003$) and at an earlier age than non-HBC patients (mean, 42.8 years and 47.1 years vs. 62.0 years, $p < 0.0001$). Basic pathology findings indicated that when compared with non-HBC, invasive BRCA1-related HBC had a statistically significantly lower diploidy rate, a lower mean aneuploid DNA index, and a strikingly higher proliferation rate. Among aneuploid tumors, S-phase fraction were higher in the BRCA1 mutation carriers. BRCA2 patients had more tubular-lobular group carcinomas ($OR = 2.56$, $p = 0.007$). All these findings were independent of age. We are now in the process of examining environmental and known endogenous risk factors which will be compared with a control group employing multiple regression analysis. Knowledge from this research will have a significant translational impact upon more common sporadic reoccurring breast cancer. Long-term, this may even impact upon DNA-targeted chemotherapy.				
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FOREWORD

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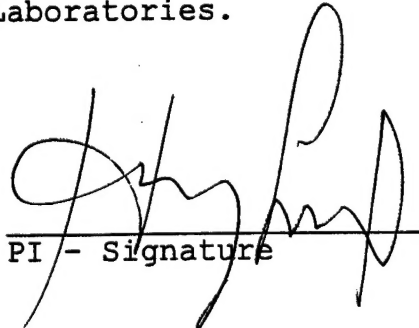
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INTRODUCTION

Despite significant advances in chemotherapy, radiation therapy and surgery, the survival rate of patients with breast cancer has shown only marginal improvement during the past several decades. Novel research studies are needed to better comprehend the etiology and pathobiology of breast cancer in the hope of translation to therapeutic benefit. BRCA1 and BRCA2 families provide extraordinary research models to test hypotheses relevant to the pathobiology and survival of breast cancer. Might BRCA2 cancers be more sensitive to radiation?¹ Are known breast cancer risk factors important in BRCA1 and BRCA2? Are there survival differences in BRCA1 and BRCA2 breast and ovarian cancers? To date, none of this risk factor information has provided satisfactory explanations as to their impact at the molecular genetic level.

Our purpose is to extend our investigation to approximately 100 families with approximately 400 breast cancer patients where slides and tissue blocks will be retrieved and medical histories including risk factors and survival parameters will be documented. Mutations within the BRCA1 and BRCA2 genes will be utilized as part of a subset analysis in an attempt to correlate phenotype with specific genotype. We postulate that mutations within BRCA1 and BRCA2 might differentially predispose breast cancer patients to specific phenotypic changes, such as differences in pathobiology and survival. These concerns are clearly cogent to a better understanding of the etiology, pathogenesis, and survival of breast cancer affected patients from BRCA1 and BRCA2 mutation positive families. This knowledge could ultimately impact upon breast cancer in the general population. It could also be important for the development of gene

therapy, particularly when considering potential differences in the phenotypic effects of mutations within BRCA1 and BRCA2.

Some evidence has shown differences in the pathobiology, and prognosis in harbingers of BRCA1 and BRCA2 mutations.² Specifically, BRCA1-related and other HBC patients both presented at lower stage ($p = 0.003$) and earlier age than non-HBC patients (mean, 42.8 years and 47.1 years vs. 62.0 years, $p < 0.0001$). Compared with non-HBC, invasive BRCA1-related HBC had a lower diploidy rate (13% vs. 35%; $p = 0.002$), lower mean aneuploid DNA index (1.53 vs. 1.73; $p = 0.002$), and strikingly higher proliferation rates (mitotic grade 3; odds ratio [OR] = 4.42; $p = 0.001$; aneuploid mean S-phase fraction 16.5% vs. 9.3%, $p < 0.0001$). Other HBC patients, including patients in two BRCA2-linked families, had more tubular-lobular group (TLG) carcinomas (OR = 2.56, $p = 0.007$). All trends were independent of age. A nonsignificant trend toward better crude survival in both HBC groups was age- and stage-dependent. Compared with non-HBC in age and stage adjusted analysis, BRCA1-related HBC patients survived no longer. Other HBC patients, despite neutral prognostic indicators, fared worse. Subsequently, a subset analysis based exclusively on BRCA1 and BRCA2 germline mutations clearly defined the pathobiology separation between BRCA1 and BRCA2.^{3,4,5}

BODY

Methods

Families ascertained in this study were classified as HBC or HBOC (Hereditary Breast Ovarian Cancer) if breast and specific other cancers (particularly ovarian) occurred in a pattern consistent with a highly penetrant, autosomal dominant disorder, especially with early ages at onset and

multiple primary or bilateral cancer diagnoses⁶. Patients were selected from families where gene linkage studies and/or germline mutations for BRCA1 and BRCA2 have been performed.

All living subjects and the next of kin of deceased subjects are asked to sign an informed consent to release clinical information, slides and tissue blocks. A risk factor questionnaire was mailed out to all subjects for completion.

Upon receipt of the signed consent form, requests for slides, tissue blocks, and clinical information of the subjects' breast cancer diagnoses are sent to the appropriate hospitals. Once slides and blocks are received, a random number is assigned then the H&E stained slides and blocks are sent to Dr. John Bishop in the Department of Pathology at Creighton University for selection of adequate specimens. Once slides are selected additional slides are cut from the accompanying blocks. Quantitative DNA flow cytometry is then performed on the blocks by Dr. Bishop. The DNA histograms will be classified and scored with regard to ploidy, DNA index and S-phase fraction, as described².

The slides are sent to Dr. Joseph Marcus and Dr. David Page for histopathological classification and grading. The two pathologists will simultaneously view the slides in a two-headed microscope and will arrive at a consensus diagnosis, using the WHO breast cancer classification⁷ with minor modifications⁸.

Significances of differences between BRCA1 and BRCA2, and among mutation-defined subgroups of BRCA1 and BRCA2, will be assessed by chi-square or Fisher's exact test or one-

way ANOVA) for frequencies) and the Mann-Whitney U test (for means of continuous variables). Logistic regression will be used to assess the effects of patient age and other co-variates on data trends.⁹ Odds ratios with 95% confidence intervals (CI) will be calculated for pathobiologic traits for BRCA1 vs BRCA2 differences.

Differences in time to death (crude death rate), time to first recurrence (recurrence rate), and time to breast cancer death (breast cancer-specific death rate) will be assessed by Cox proportional hazards survival analysis¹⁰, using EGRET software (Statistics and Epidemiology Research Corporation, Seattle, WA). Hazards ratios (HR) of BRCA1 vs. BRCA2, with 95% confidence intervals, will be calculated for regression terms. S. Narod, M.D., will perform risk factor analysis and will assist with survival analysis.

RESULTS AND DISCUSSION

Drs. Marcus & Narod traveled to Creighton University in February 1998 to confer with Dr. Lynch and his colleagues regarding the organization of this study. Our efforts during the first year were targeted at identifying qualifying families, organizing demographic data on all breast cancer cases, and developing a database to collect the information in a systematic and efficient manner.

Forty-eight HBC or HBOC families have been identified and targeted for this research study. Within these families, 211 breast cancer cases have been identified (these cases were not part of the preliminary study done in 1996). To date 176 cover letters have been sent out to breast

cancer affected individuals or to the legal next of kin of deceased individuals in order to obtain a signed informed consent for slides, tissue blocks, and clinical information retrieval. Since the time the cover letters were mailed out in the beginning of June, 52 signed consent forms have been returned.

Four cases of slides and paraffin tumor blocks have been collected to date for Dr. Bishop's selection of the best blocks (with the most tumor cross section) and for cutting of additional slides for the marker bank and DNA flow cytometry studies.

Risk factor questionnaires have been completed and returned to our center by 102 breast cancer affected individuals. These questionnaires have been forwarded to Dr. Steven Narod for data entry and assessment of survival parameters.

REFERENCES

1. D. W. Abbott, M.L. Freeman, J.T. Holt, *J. Natl. Cancer Inst.* **90**, 978-985 (1998).
2. J.N. Marcus, P. Watson, D.L. Page, S.A. Narod, G.M. Lenoir, P. Tonin, L. Linder-Stephenson, G. Salerno, T. Conway, H.T. Lynch, *Cancer* **77**, 697-709 (1996).
3. J.N. Marcus, D.L. Page, P. Watson, S.A. Narod, G.M. Lenoir, H.T. Lynch, *Cancer* **80**, 534-556 (1997).
4. J.N. Marcus, P. Watson, D.L. Page, S.A. Narod, P. Tonin, G.M. Lenoir, O. Serova, H.T. Lynch, *Breast Cancer Res. Treat.* **44**, 275-277 (1997).
5. P. Watson, J.N. Marcus, H.T. Lynch, *Lancet* **351**, 304-305 (1998).
6. H.T. Lynch, J.N. Marcus, P. Watson, J. Lynch, in *The Breast*, K. Bland and E.M. Copeland, Eds. (W.B. Saunders Co., Philadelphia, 1991), pp. 262-291.
7. The World Health Organization, *Am. J. Clin. Path.* **78**, 806-816 (1982).
8. D.L. Page, T.J. Anderson, G. Sakamoto, in *Diagnostic Histopathology of the Breast*, D.L. Page and T.J. Anderson, Eds. (Churchill Livingstone, Edinburgh, 1987), pp. 193-235.
9. S.H. Walker and D.B. Duncan, *Biometrika* **54**, 167-179 (1967).

10. J.D. Kalbfleisch and R.L. Prentice in *The statistical analysis of failure time data*, J.D.

Kalbfleisch and R.L. Prentice, Eds. (John Wiley & Sons, New York, 1980), pp. 70-117.